15

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
23388P WO		
International application No.	International filing date *(day month year)*	Priority date *(day month year)*
PCT/EP00/00623	27/01/2000	28/01/1999
International Patent Classification (IPC) or national classification and IPC A61K38/57		
Applicant DONY, CAROLA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.08.2000	29.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00623

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-16 as originally filed

Claims, No.:

1-17 with telefax of 02/01/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 16-17 (industrial applicability).

because

☒ the said international application, or the said claims Nos. 16-17 (industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-15; 16-17 see separate sheet

No: Claims -

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1- Claims 16-17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2- Reference is made to the following document:

D1: WO 95 03328 A (BUETTNER REINHARD ;BOGDAHN ULRICH (DE); KALUZA BRIGITTE (DE); BOEH) 2 February 1995 (1995-02-02) cited in the application

NOVELTY - Art. 33 (1) and (2) PCT

3- Claims 1-17 appear to be novel in the light of the prior art cited in the search report:

3.1- The novel features are:

- a pharmaceutical composition containing a MIA factor and a biocompatible and/or biodegradable matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen or combinations of these,
- the combination of MIA with an osteoinductive protein, and
- the use of MIA for bone and or cartilage repair.

3.2- D1 merely discloses the therapeutic use of MIA in combination with a filler (p 11 § 1-

3) as well as the use of the MIA gene sequence for gene therapy by means of a vector (p 11 § 4) but does not mention bone or cartilage repair.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

4- Claims 1-17 appear to be inventive for the following reasons:

4.1- The problem posed in the present application is to provide therapeutic means for the induction of bone and/or cartilage repair.

4.2- The solution proposed in the present application is the use of MIA.

4.3- D1 discloses the use of MIA for the treatment of tumors.

D1 is considered to be the closest prior art.

D1 neither discloses nor suggests a pharmaceutical composition containing a MIA factor and a biocompatible and/or biodegradable matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen or combinations of these and/or the combination of MIA with an osteoinductive protein and/or the use of MIA for the induction of bone and/or cartilage repair.

4.4- Therefore, claims 1-17 can be considered as being inventive.

INDUSTRIAL APPLICABILITY - Art. 33 (1) and (4) PCT

5- Claims 1-15 appear to be industrially applicable.

6- For the assessment of the present claims 16-17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The

patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

7- Claim 12, as a claim dependent on claim 11 is wrongly drafted as "a method" and should read as "a use" (Rule 6 PCT).

International Application
No.PCT/EP00/00623
Dr.Carola Dony

New Claims

1. A pharmaceutical composition containing a melanoma inhibiting activity factor and a biocompatible and/or biodegradable matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen, or combinations of these.

2. A pharmaceutical composition containing a melanoma inhibiting activity factor (MIA) in combination with an osteoinductive protein.

3. A pharmaceutical composition as claimed in claim 2, wherein the ratio of osteoinductive protein : MIA is 1 : 1 to 1 : 20.

4. A pharmaceutical composition as claimed in claim 2 or 3, wherein the osteoinductive protein is BMP-2, BMP-7 or a hedgehog protein.

5. A pharmaceutical composition as claimed in claims 2 to 4, wherein the composition includes a biocompatible matrix.

6. A pharmaceutical composition as claimed in claim 1 or 5, wherein the biocompatible matrix is hyaluronic acid, alginate, collagen, heparin, polylactic-coglycolid and/or polylactic-coglycolid derivatives or combinations thereof.

7. Use of a melanoma inhibiting activity factor (MIA) as the essential component for manufacturing a pharmaceutical composition for improved induction of the chondro-/osteogenic lineage and promotion of cartilage and/or bone formation.

8. A use according to claim 7, wherein the composition contains in addition an osteoinductive protein.

9. A use as claimed in claim 8, wherein the osteoinductive protein is BMP-2 or BMP-7 or a hedgehog protein.

10. A use as claimed in claim 8 or 9, wherein the ratio of osteoinductive protein : MIA is 1 : 1 to 1 : 20.

11. A use as claimed in claims 8 to 10, wherein the melanoma inhibiting activity factor (MIA) is combined with a biocompatible matrix.

12. A method as claimed in claim 11, wherein the biocompatible matrix is hyaluronic acid, alginate, collagen, heparin, polylactic-coglycolid and/or polylactic-coglycolid derivatives or combinations thereof.

13. Use of an expression vector for a melanoma inhibiting activity factor (MIA) or a combination of a vector for the expression of an osteoinductive protein with a vector capable of expression of a melanoma inhibiting activity factor (MIA) for manufacturing a pharmaceutical composition for improved induction of the chondro-/osteogenic lineage and promotion of cartilage and/or bone formation.

14. A use of an expression vector capable of expression of a melanoma inhibiting activity factor (MIA) or a vector capable of expression of an osteoinductive protein and a vector capable of expression of a melanoma inhibiting activity factor (MIA) as essential component for manufacturing a pharmaceutical composition for improved induction of the chondro-/osteogenic lineage and promotion of cartilage and/or bone formation.

15. A use as claimed in claim 13, wherein the composition includes a biocompatible matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen, or combinations of these.

16. The use of a melanoma inhibiting activity factor (MIA) for the treatment of a patient in need of bone and/or cartilage repair.

17. The use according to claim 18, wherein a combination of a melanoma inhibiting activity factor (MIA) and an osteoinductive protein is used.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C. 20231
ETATS UNIS D'AMERIQUE

Date of mailing (day/month/year)	
09 October 2000 (09.10.00)	
International application No	**Applicant's or agent's file reference**
PCT EP00 00623	Case 20311
International filing date (day/month/year)	**Priority date** (day/month/year)
27 January 2000 (27.01.00)	28 January 1999 (28.01.99)
Applicant	
DONY, Carola et al	

1. The designated Office is hereby notified of the election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

25 August 2000 (25.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer G. Bahr
Facsimile No. +41 22 740 14 35	Telephone No. +41 22 338 83 38

Form PCT/IB/331 (July 1992)

EP/000623

PATE COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To: 13 Rec'd PCT/PTO 2001
09/806635

WEICKMANN & WEICKMANN
Kopernikusstrasse 9
D-81679 Munchen
ALLEMAGNE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day month/year)
31 August 2001 (31.08.01)

Applicant's or agent's file reference
Case 20311

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/00623

International filing date (day month year)
27 January 2000 (27.01.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
LESER, Ulrike
Elisabethstrasse 26
D-80796 München
Germany

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
3-00 LESER-REIFF, Ulrike
Elisabethstrasse 26
D-80796 München
Germany DE X

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Gabriele BAEHR

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

WEICKMANN & WEICKMANN
Kopernikusstrasse 9
D-81679 Munchen
ALLEMAGNE

Date of mailing (day/month/year) 12 September 2000 (12.09.00)	
Applicant's or agent's file reference Case 20311	**IMPORTANT NOTIFICATION**
International application No. PCT EP00 00623	**International filing date** (day month year) 27 January 2000 (27.01.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
SCHREINER, Siegfried Roche Diagnostics GmbH Patent Department Pharma (TR-E) P.O. Box 11 52 D-82372 Penzberg Germany		

Telephone No.: 08856 60 34 46

Facsimile No.: 08856 60 34 51

Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
WEICKMANN & WEICKMANN Kopernikusstrasse 9 D-81679 Munchen Germany		

Telephone No.: 089 45563 0

Facsimile No.: 089 45563 999

Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office [X] the designated Offices concerned

[ ] the International Searching Authority [ ] the elected Offices concerned

[ ] the International Preliminary Examining Authority [ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer G. Bahr
Facsimile No.: +41 22 740 14 35	Telephone No.: +41 22 338 83 38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

WEICKMANN & WEICKMANN
Kopernikusstrasse 9
D-81679 München
ALLEMAGNE

20. SEP. 2000

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)	
12 September 2000 (12.09.00)	
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
Case 20311	
International application No.	**International filing date** (day/month/year)
PCT/EP00/00623	27 January 2000 (27.01.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
F. HOFFMANN-LA ROCHE AG CH-4070 Basle Switzerland	CH	CH
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
DONY, Carola Engelstrasse 7 D-81477 München	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer: G. Bahr
Facsimile No.: (41-22) 740 14 35	Telephone No.: (41-22) 338 83 38

Form PCT/IB/306 (March 1994) 0038184[illegible]

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Weickmann Weickmann Prechtel Weiss
Tiesmeyer Herzog Böhm Liska & Huber
Kopernikusstrasse 9
81679 München
ALLEMAGNE

03. JAN. 2001

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing (day/month/year)	29.12.2000
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
23358P WO	

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/EP00/00623	27/01/2000	28/01/1999

Applicant
DONY, CAROLA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hundt, D Tel. +49 89 2399-8141

Form PCT/IPEA/416 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
23388P WO		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT EP00/00823	27/01/2000	28/01/1999

International Patent Classification (IPC) or national classification and IPC
A61K38/57

Applicant
DONY, CAROLA et al.

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   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

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   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/08/2000	29/01/2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 8180

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00623

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17))*:
   Description, pages:

   1-16 as originally filed

   Claims, No.:

   1-17 with telefax of 02/01/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
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   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
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   - ☐ the description, pages:
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 16-17 (industrial applicability).

because:

☒ the said international application, or the said claims Nos. 16-17 (industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-17
	No:	Claims -
Inventive step (IS)	Yes:	Claims 1-17
	No:	Claims -
Industrial applicability (IA)	Yes:	Claims 1-15; 16-17 see separate sheet

No Claims -

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1- Claims 16-17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2- Reference is made to the following document:

D1: WO 95 03328 A (BUETTNER REINHARD ;BOGDAHN ULRICH (DE); KALUZA BRIGITTE (DE); BOEH) 2 February 1995 (1995-02-02) cited in the application

NOVELTY - Art. 33 (1) and (2) PCT

3- Claims 1-17 appear to be novel in the light of the prior art cited in the search report:

3.1- The novel features are:

- a pharmaceutical composition containing a MIA factor and a biocompatible and/or biodegradable matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen or combinations of these,
- the combination of MIA with an osteoinductive protein, and
- the use of MIA for bone and/or cartilage repair.

3.2- D1 merely discloses the therapeutic use of MIA in combination with a filler (p 11 § 1-

3) as well as the use of the MIA gene sequence for gene therapy by means of a vector (p 11 § 4) but does not mention bone or cartilage repair.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

4- Claims 1-17 appear to be inventive for the following reasons:

4.1- The problem posed in the present application is to provide therapeutic means for the induction of bone and/or cartilage repair.

4.2- The solution proposed in the present application is the use of MIA.

4.3- D1 discloses the use of MIA for the treatment of tumors.

D1 is considered to be the closest prior art.

D1 neither discloses nor suggests a pharmaceutical composition containing a MIA factor and a biocompatible and/or biodegradable matrix selected from the group consisting of hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen or combinations of these and/or the combination of MIA with an osteoinductive protein and/or the use of MIA for the induction of bone and/or cartilage repair.

4.4- Therefore, claims 1-17 can be considered as being inventive.

INDUSTRIAL APPLICABILITY - Art. 33 (1) and (4) PCT

5- Claims 1-15 appear to be industrially applicable.

6- For the assessment of the present claims 16-17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The

patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

7- Claim 12, as a claim dependent on claim 11 is wrongly drafted as "a method" and should read as "a use" (Rule 6 PCT).

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(54) Title: USE OF A MELANOMA INHIBITING ACTIVITY FACTOR (MIA) FOR CARTILAGE AND BONE REPAIR

(57) Abstract

A melanoma inhibiting activity factor (MIA), preferably in combination with an osteoinductive protein, is a useful pharmaceutical agent for promoting bone healing and/or cartilage repair.

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Use of a melanoma inhibiting activity factor (MIA) for cartilage and bone repair

The present invention relates to a method and a composition for the induction of the chondro-/osteogenic lineage from mesenchymal stem cells and for promoting cartilage and bone formation using a melanoma inhibiting activity factor (MIA) preferably in combination with an osteoinductive protein.

MIA was initially described as a factor inhibiting the growth of malignant melanoma cell line HTZ-19 (Weilbach et al., Cancer Res. 50 (1990) 6981-6986). Cloning and purification of the factor resulted in a novel 11 kD protein with anti-tumor activity (WO 95/03328). The bovine homolog CD-RAP (cartilage derived-retinoic acid-sensitive protein) was detected in cartilage primordia and cartilage (Dietz, U., and Sandell, L., J. Biol. Chem. 271 (1996) 3311-3316). The mouse CD-RAP/MIA gene was localized in embryonic mouse cartilage and the transcripts were detected in chondrosarcomas (Bosserhoff et al., Developmental Dynamics 208 (1997) 516-525). These data point to a normal expression of MIA in cartilage. Further data are derived from transgenic mice where MIA promoter directs the cartilage specific expression of lacZ (Xie et al., 44th Annual Meeting, Orthopaedic Research Society, March 16-19, 1998, New Orleans, Louisiana). MIA could also be used as a progression marker for malignant melanoma (Bosserhoff et al., Cancer Research 57 (1977) 3149-3153; DE 196 53 358 A1)

Osteoinductive proteins are proteins which induce the full developmental cascade of endochondral bone formation towards chondrocytes and osteocytes and are, for example, hedgehog proteins (Sonic (Shh), Indian (Ihh), Desert (Dhh); Kinto et al., Kinto et al., FEBS Letters 404 (1997) 319-323), or members of the bone morphogenetic protein family (BMPs).

Hedgehog proteins, especially sonic hedgehog (Shh) are responsible for the development of multiple organ systems, including brain, spinal cord, craniofacial structures, limbs, the eye, left and right body symmetry, somite patterning (Hammerschmidt et al., Trends Genet. 13 (1997) 14-21). Indian hedgehog (Ihh) plays a role in cartilage development (Vortkamp et al., Science 273 (1996) 613-622; Lanske et al., Science 273 (1996) 663-666). Desert hedgehog (Dhh) is involved in the development of male germ line cells. Further evidence for involvement of hedgehog, e.g. Shh, in bone development and repair is given by mutations leading

to human holoprosenphaly (Roessler et al., Human Molecular Genetics 6 (1997) 1847-1853; Belloni et al., Nature Genetics 14 (1996) 353) and by the induction of ectopic bone after expressing Shh in fibroblasts and transplantation of the cells in muscles (Nakamura et al., BBRC 237 (1997) 465-469); Kinto et al., FEBS Letters 404 (1997) 319-323).

Bone morphogenetic proteins (BMPs) are molecules which are responsible for the formation of bone, cartilage, tendon, and other tissues, shown by ectopic bone formation (Wozney et al., Science 272 (1988) 738-741). The unique inductive activities of these proteins, along with their presence in bone, suggest that they are important regulators of bone repair processes and may be involved in the normal maintenance of bone tissue. Many such proteins are known which can be divided into several sub-families (Reddi, A.H., Cytokine & Growth Factor Reviews 8 (1997) 11-20). Such BMPs are, for example, BMP-2 to BMP-14 and the growth and developmental factors GDF-1 to GDF-14.

BMPs are important signaling factors and regulate the multistep sequential cascade in bone and cartilage formation such as chemotaxis, mitosis and differentiation. Especially, BMP-2, BMP-3, BMP-4, BMP-5, BMP-7 initiate chondrogenesis and osteogenesis.

In the case of promoting bone healing, only limited success has been achieved. Currently, large bone defects (orthopedic reconstruction) are treated with either bone or bone powder grafting either autografts or allografts. In addition, in all cases of bone fractures about 5-10% show difficulty in healing, either delayed union (healing only after 6 month) or no healing (non-union still after 9 month) (Einhorn, T.A., Journal of Bone and Joint Surgery, American Volume 77A (1995) 940-956). Allograft bone and bone powder are derived from human donors and can be stored in bone tissue banks, but are limited. Since it is human material, extensive screening for viral (e.g. HIV, HBV, HCV) and bacterial contamination is necessary. Also graft rejections may occur. The material varies in quality depending on donor. The use of autologous bone is often accompanied by morbidity at the graft site (Muschler et al., Clin. Orthop. Rel. Res. (1996) 250-260). In addition there is only a limited amount of such a material available from the autologous donor.

Clinical trials for BMP-2 and BMP-7 alone to promote bone healing have been started. The first results indicate that BMP-2 or BMP-7 seem to be equivalent to bone or bone powder grafts (Boyne, J. Oral Maxillofac. Surg. 53 Suppl 4 (1995) 92; Kirker-Head et al., Clin. Orthop. 218 (1995) 222; Johnson et al., Clin. Orthop. 277 (1992) 229). About 2.5 to 6.8 mg per g matrix are used.

There is a high medical need for improved and enhanced cartilage repair. Current therapies for acute defects (e.g. car or sport accidents), either partial thickness, full thickness or gap defects, are excision, debridement or waiting for very rarely occurring self-healing. There are some therapies under investigation, e.g. mosaic plastic, using autogenous bone/cartilage graft in the shape of a cylinder for large defects. There are a few cell therapy approaches in preclinical and premarketing studies. Autologous chondrocytes isolated during a biopsy are cultivated in vitro as a monolayer (Brittberg et al., N. Engl. J. Med. 331 (1994) 889-895). The dedifferentiated cells are injected under a periosteal flap sutured over the defect in an open knee surgery. Mesenchymal stem cells are in preclinical studies which can differentiate into chondrocytes on an appropriate carrier (US-P 5,486,359). There exists no easy-to-use therapy yet using a protein or combinations of proteins.

WO 98/30234 describes a composition of BMP and hedgehog proteins. WO 97/21447 describes a combination of osteoinductive bone morphogenetic protein (e.g., BMP-7) and a morphogenetic protein stimulating factor IGF-1 for bone healing. WO 92/09697 describes a combination of BMP and TGF-ß for such purposes. Factors healing cartilage either alone or in combination are described in WO 96/14335 (cartilage derived morphogenetic proteins) and WO 97/23612.

Further combinations of factors for bone healing are described in US-P 5,270,300: osteogenic factor (TGF-beta, TGF-beta and EGF, osteogenin, BMP, + combinations thereof) and angiogenic factor (TGF-beta, angiogenin, angiotropin, FGF-2, PDGF-a and combinations thereof) for bone healing; in US-P 5,629,009: TGF-beta, EGF, or factors derived from demineralized bone matrix (between about 10 and 90 % by weight of matrix) combined with FGF or PDGF; in EP-B 0 429 570 by Genetics Institute, Inc.: combination of BMPs (protein or DNA) with different type of carriers. There are also mentioned combinations of BMPs with EGF, FGFs, PDGF, TGF-alpha and TGF-beta.

The invention provides a method for improved induction of the chondro-/osteogenic lineage and promoting cartilage and enhanced bone formation, using MIA, preferably in combination with an osteoinductive protein.

The invention further relates to a method for manufacturing a pharmaceutical composition for induction of the chondro-/osteogenic lineage and the promotion of cartilage and bone formation, wherein a melanoma inhibiting activity factor (MIA) according to the invention is used as an essential component of this pharmaceutical composition. It is further preferred to use a combination of MIA and an osteoinductive protein as essential components. The ratio of osteoinductive protein : MIA is preferably 1 : 1 to 1 : 20.

It was surprisingly found that MIA, preferably in combination with an osteoinductive (osteogenic) protein, preferably with a bone morphogenetic protein 2, 3, 4, 5 or 7 or a hedgehog protein, results in cartilage and/or bone formation.

By "osteoinductive protein" is preferably understood an osteogenic protein which induces endochondral bone formation. Chondrocytes produce cartilageneous matrix followed by osteoblasts and osteocytes which produce bone tissue. Early genes of the chondro-/osteogenic lineages, e.g. Cbfa1, are thereby upregulated, and this ultimately leads to the formation of chondrocytes and osteocytes. Such an osteoinduction can be achieved, for instance, through BMPs or hedgehog proteins. BMP-2, BMP-7, or hedgehog protein (Shh, Ihh or Dhh) is preferred. The osteoinductive proteins useful in this invention include also proteins such as TGF-ß, BMPs, and TGF-ß combined with EGF.

A substance's ability to induce osteogenesis can be tested in a simple manner. For this purpose, for example, pluripotent mesenchymal cells, e.g., C3H10T1/2 cells, are cultured with and without the potential osteoinductive factor. Controls and treated cells are measured for alkaline phosphatase activity. The activity can be measured photometrically using a suitable colorimetric substrate, e.g., p-nitrophenyl phosphate (Nakamura et al., BBRC 237 (1997) 465-469). Increased activity of alkaline phosphatase is scored as osteoinduction. Alternatively, upregulation of osteocalcin and alkaline phosphatase is measured by RT-PCR using suitable primers for osteocalcin and alkaline phosphatase.

A compound's ability to induce chondrogenesis can be tested in vitro using pluripotent mesenchymal cells, e.g. C3H10T1/2 or pre-chondrogenic cells, e.g. RCJ3.1C5.18. The cells are cultivated in three-dimensional cultures, e.g. micromass culture with the inductor or a combination of inductors for two to three weeks. Collagen type II as cartilage marker could be proven either by immunocytochemistry using monoclonal antibodies or by Northern blot after RNA isolation. Alcian blue staining proves the existence of proteoglycans. A different method would be to test for aggrecan using specific primers in RT-PCR reaction.

In a further preferred embodiment of the invention, MIA, preferably in combination with an osteogenic protein, can be introduced in the cells via gene therapy methods ex vivo or in vivo. For this method the genes coding for MIA, and optionally, for the osteogenic protein are introduced in one vector, preferably under the control of the same promoter, or in separate vectors. For an efficient expression of MIA and the osteogenic protein, it is necessary to use strong promoters in the vectors. Such promoters are, e.g., PGK or CMV promoters. Preferably, the expression vector consists of such a strong promoter, the full-length mRNA of the chosen gene, e.g., BMP-2, BMP-3, BMP-4, BMP-5, BMP-7, Shh, Ihh, or Dhh, FGF, HGF, PlGF, VEGF, an artificial intron and a poly-A-site. For in vivo application, DNA is either lyophilized to collagen sponges, preferably for osteogenesis, or applied with any other suitable carrier, preferably hyaluronic acid or collagen for application as a gel for chondrogenesis. For ex vivo application, cells of the chondrogenic and osteogenic lineage are transfected with such vectors and subsequently implanted.

The pharmaceutical formulation according to the invention may also include an appropriate matrix, for instance, for delivery and/or support of the composition and/or providing a surface for bone formation. The matrix may provide slow release of MIA, preferably in combination with an osteoinductive protein. Slow release for MIA is possible by combining MIA with a matrix to which MIA is bound in a reversible manner by ionic or hydrophobic interaction. Preferably, the composition includes a matrix which is biocompatible and/or biodegradable. Potential matrices for the compositions contain, for example, hyaluronic acid, alginate, calcium sulfate, tricalcium phosphate, hydroxylapatite, polylactic-coglycolid, polyanhydrides, collagen, or combinations of these, whereby hyaluronic

acid, alginate, heparin, collagen and/or polylactic-coglycolid or derivatives thereof are preferred.

For local bone repair, it is preferred to use MIA or its combination with the osteoinductive protein. It is therefore preferred to use for osteogenesis form-stable matrices in close contact with the progenitor cells. MIA or the combination applied to a three-dimensional matrix like a sponge and put tightly into the defect enable cells, e.g. from periost or bone marrow, to proliferate and differentiate into bone cells which are preferably biodegradable. Preferred materials for such sponges are, for example, collagen, alginate, tricalcium phosphate, hydroxylapatite and combinations thereof.

For the induction of chondrogenesis, it is essential that MIA or its combination with the chondrogenic/osteogenic protein should be directed to the local cartilage defect. Cartilage progenitor cells are derived either from the subchondral bone (in full thickness defects) or from the synovial membrane (in partial thickness defects). The treatment enables the cells to proliferate and to differentiate which results in the synthesis of new cartilage. Mature chondrocytes from the surrounding area could be stimulated, too. To this end, it is expedient that the pharmaceutical composition should be applied directly onto, or into, the cartilage tissue, preferably by local implantation or local injection. Suitably, this is done by means of a syringe. Here, again, the use of a matrix is preferred. However, it is preferred that this matrix, rather than being form-stable, should be flowable like a gel or a paste. Preferably, the flowability is high enough to allow the pharmaceutical formulation to be applied with a syringe.

The dosage regimen will be determined by the attending physician, considering various facts which modify the action of the formulation of the invention. Factors which may modify the action of the formulation include the amount of bone desired to be formed, the site of application, the condition of the damage, the patient's age, sex and diet, the severity of any infection, time of administration, and other clinical factors. The dosage may vary with the type of the matrix used in the reconstitution of bone.

The invention further relates to a process for the production of a pharmaceutical agent which is characterized in that MIA is used as an essential component of this

agent. In this process, it is preferred to use 500 µg of MIA per implant or per bolus injection. In a preferred embodiment, the pharmaceutical agent contains in addition an osteoinductive protein. The weight ratio of osteoinductive protein : MIA is preferably 1 : 1 to 1 : 20. It is thus preferred to use an excess amount of MIA. In this composition, it is preferred to use about 100 µg of osteoinductive protein and about 500 µg of MIA. The overall amount of MIA and osteoinductive protein is preferably in the range between 200 and 800 µg, referred to gram of matrix protein.

For the cartilage applications, such a pharmaceutical formulation is preferably a gel based on a hyaluronic or collagen matrix. Such a gel is preferably injectable and is applied in an amount of 100 µl to 2 ml per bolus injection. In the case of application in the bone, the use of a collagen sponge is preferred.

The invention further relates to a pharmaceutical composition of this kind. A pharmaceutical composition of this kind can be applied for bone repair, osteogenesis in vivo, especially for the treatment of patients who suffer from bone defects and hence are in need of bone repair as well as for cartilage repair.

A further object of the invention is a pharmaceutical composition containing an expression vector for MIA, and optionally, in addition, for an osteoinductive protein, or a combination of a vector for the expression of MIA with a vector capable of expression of an osteoinductive protein, as well as a method for manufacturing such a pharmaceutical composition.

The following examples and references are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Example 1

In vitro cell assay for induction of osteogenic differentiation

Mesenchymal cells, e.g. C3H10T1/2 cells are seeded into 96 well plates. After 24 hours, the osteoinductive factor, e.g. hedgehog or BMP, is added alone or in combination with MIA (see Table 1). For control, cells are untreated. After 5 days

control and treated cells are analyzed for alkaline phosphatase activity and protein content. Alkaline phosphatase (AP) activity is measured photometrically using p-nitrophenyl phosphate as a colorimetric substrate. Increase in activity is scored as osteoinduction. For hedgehog 0.05 µg/ml was applied. MIA was tested in various concentrations from 0.05 µg/ml to 50 µg/ml.

MIA applied alone did not change the alkaline phosphatase activity. When MIA was applied in combination with hedgehog a synergistic effect was observed resulting in 2.7 fold increase of alkaline phosphatase activity.

Table 1

Factor	µg/ml	mmol PNP/min/mg protein	% of control
Hedgehog	0.05	14.43	309
MIA	50	4.26	91
MIA	10	3.85	83
MIA	5	4.14	89
MIA	1	3.98	85
MIA	0.5	3.71	79
MIA	0.1	3.77	81
MIA	0.05	4.86	104
Hedgehog + MIA	0.05 + 50	39.23	839
Hedgehog + MIA	0.05 + 10	26.60	569
Hedgehog + MIA	0.05 + 5	30.57	654
Hedgehog + MIA	0.05 + 1	16.11	345
Hedgehog + MIA	0.05 + 0.5	20.08	429
Hedgehog + MIA	0.05 + 0.1	25.09	536
Hedgehog + MIA	0.05 + 0.05	21.09	451
negative control		4.67	100

Example 2

In vitro assay for induction of cartilage markers

Chondrocytes of pigs were isolated from femoral condyles. Primary human chondrocytes were isolated from femoral condyles of patients undergoing knee surgery. The cartilage was minced into small pieces and incubated in 10 ml with 2 mg/nl of collagenase (Roche Diagnostics GmbH, DE) and 0.1 mg/ml of hyaluronidase (Sigma) and 0.15 mg/ml DNase (Roche Diagnostics GmbH, DE) for 16 h at 37°C. After centrifugation, the chondrocytes were seeded in petri dishes for proliferation.

The dedifferentiated cells were used for assays. 2×10^4 cells in 10 µl medium were spotted per well in 96-well plates. After 4 h, 200 µl medium were added. After 7 days, inductors were added to the micromass culture: BMP-2, hedgehog, MIA, and combinations thereof. Two to four weeks later, the cultures were assayed for cartilage markers. Morphologically, chondrocytes are visible by their round appearance. Immunocytochemistry shows collagen type II expression. Cytochemically, Alcian blue proves sulfated proteoglycans. With PCR, aggrecan and SOX9 could be shown.

Example 3

In vitro assay for induction of proliferation

Chondrocytes were isolated from the femoral condyles of pigs. 3,000 cells were seeded in 96 well plates and cultivated for 3 days. After 24 h of serum-free incubation, MIA, BMP-2, Shh and combinations thereof were added. During the last 16 h of the 48 h serum-free induction period, BrdU labeling was present. The detection ELISA was done according to the instructions of the manufacturer (Roche Diagnostics GmbH).

Table 2

factor	ng/ml concentration	% stimulation above serum-free control
hedgehog	100	88
	50	93
BMP-2	500	112
	100	69
MIA	50,000	195
	10,000	85
	2,000	99
MIA + BMP-2	50,000 + 500	125
	10,000 + 500	237
	50,000 + 100	203
	10,000 + 100	133
MIA + hedgehog	50,000 + 100	115
	10,000 + 100	224
	50,000 + 50	261
	10,000 + 50	131
fetal calf serum		792
serum-free control		100

MIA alone and in combination stimulates DNA synthesis of primary chondrocytes.

Example 4

In vitro organ assay to study chondrogenesis: mouse limb bud assay

Limb buds are isolated from E12.5 to E15.5 mouse embryos (NMRI) using microdissecton scissors and watchmaker's forceps under sterile conditions. The limb buds were rinsed in PBS containing an antibiotic-antimycotic from Gibco-BRL (#15240-039), then cultured in serum-free BGJb medium from Gibco-BRL (#12591-020) for 48 h to 144 h in organ culture dishes. After 24 h of culture MIA, BMP-2 alone or various combinations of MIA and BMP were added. Media were changed every day. At the end of the culture the limbs were rinsed in PBS, then fixed overnight in 4% paraformaldehyde, either processed for paraffin embedding

or for wholemount in situ hybridization as described by Wilkinson, D.G., In situ hybridization: a practical approach, In: Rickwood D, Hames BD (eds.) The practical approach series, Oxford Univ. Press, Oxford, New York, Tokyo (1992). Paraffin sections were stained with von Kossa to visualize and quantitate the amount of calcified areas, stained with Alcian blue to assess chondrogenesis. In addition in situ RNA hybridization was performed to analyze gene expression characteristic for cartilage development, e.g. collagen II, MIA, collagen X.

Example 5

Mouse bioassay for cartilage, bone, tendon and ligament induction

Similar to the Sampath and Reddi rat ectopic implant assay, a mouse ectopic implant assay, using inbred C3H mice, 4 months old was performed (Sampath and Reddi, Proc. Natl. Acad. Sci. USA 80 (1983) 6591-695; WO 95/16035). (a) MIA alone, (b) BMP-2 alone and (c) combinations of MIA and BMP-2 were applied in the appropriate buffer, 0.1% trifluoroacetic acid for BMP-2 and 100 mM potassium-phosphate, 150 mM NaCl, pH 6.0 for MIA. As carrier were used collagen type I matrix and hyaluronic acid. Any suitable carrier maybe used, e.g. collagen type I matrix, collagen-heparin mixture, gelatin capsules, hyaluronic acid, alginate or other functionally equivalent device, based on biocompatibility, biodegradability, stability and mechanical properties.

The implants were placed intramuscular into the gluteus muscle of the mouse and left for 14 days. After 14 days the mice were sacrificed by cervical dislocation. The implants were isolated and processed using standard histological techniques (see Theory and Practice of Histological Techniques, ed. Bancroft and Stevens, Churchill Livingstone, 1996). Paraffin sections (4 µm) were cut and stained with von Kossa to visualize and quantitate the amount of cartilage and bone tissue induced in each implant. Positive (e.g. BMP-2) and negative (e.g. mock device) implant control groups were compared to experimental implants.

To assess the quality of cartilage and/or bone induced, gene expression can be studied by RNA in situ hybridization for cartilage and bone markers as described above, using cartilage markers (e.g. collagen II, collagen X) and bone markers (e.g. collagen I, osteocalcin).

Example 6

Mouse bioassay for cartilage, bone, tendon and ligament induction for DNA expression vectors

Similar to the Sampath and Reddi rat ectopic implant assay, a mouse ectopic implant assay, using e.g. outbred NMRI mice or inbred C3H mice, 2 months old was performed (Sampath and Reddi, Proc. Natl. Acad. Sci. USA 80 (1983) 6591-695; WO 95/16035. Expression vectors for (a) osteoinductive factor alone, (b) MIA alone and (c) combinations of osteoinductive factor and MIA were lyophilized in the appropriate buffer, e.g. TE-buffer (Fang et al., Proc. Natl. Acad. Sci. USA 93 (1996) 5753-5758). Any suitable carrier may be used, e.g. collagen type I matrix, collagen-heparin mixture, gelatin capsules, hyaluronic acid, alginate or other functionally equivalent device, based on biocompatibility, biodegradability, stability and mechanical properties.

The implants were set intramuscular into the hindlimb muscle of the mouse for seven and 14 days. After seven and 14 days the mice were sacrificed by cervical dislocation. The implants were isolated and processed using standard histological techniques (see Theory and Practice of Histological Techniques, ed. Bancroft and Stevens, Churchill Livingstone, 1996). Paraffin (4 µm) sections can be stained with Toluidine Blue, Alcian Blue, von Kossa, Movat or Hematoxylin/Eosin to visualize and quantitate the amount of tendon, ligament, cartilage and bone tissue induced in each implant. Positive (e.g. BMP-2, shh expression vector) and negative (e.g. mock device) implant control groups are compared to experimental implants.

To assess the quality of cartilage and/or bone induced, gene expression can be studied by RNA in situ hybridization for cartilage and bone markers as described above.

Example 7

Non-union fracture model in rabbits (radius osteotomy)

A non-union defect of 1.5 cm in length was produced at the radius of adult rabbits in order to assess the ability of the combinations of MIA alone and MIA in combination with BMP or hedgehog proteins and appropriate carrier to affect bone repair. The animals were anesthetized by intravenous injection of

xylazine/ketamine, and surgery was carried out under sterile conditions. The defect was either left empty, filled with the appropriate carrier, or filled with a carrier containing MIA and BMP, or each of these factors alone. Animals were allowed to move freely and X-rays were carried two and four weeks after surgery in order to assess the rate of bone defect healing. At the end of study, the animals were killed under anesthesia and the bone defect site was removed for histological examination using the von Kossa and Goldner stain so as to quantify and characterize the quality of newly formed repair tissue.

Example 8

Full thickness articular cartilage repair model

A full thickness articular cartilage defect model in the femoral-patellar joint of adult rabbits is used to assess the ability of MIA alone or in combination with BMP or hedgehog protein and carrier to affect cartilage and bone repair. Adult rabbits are anesthetized and prepared for sterile surgery. An up to 4 x 4 mm defect through articular cartilage and into underlying subchondral bone is drilled into the patellar groove of the knee joint. The defect is either left empty, filled with the appropriate carrier, or filled with a carrier containing MIA alone or in combination with BMP or hedgehog protein. Animals are allowed to move freely for four weeks. After four weeks the animals are humanely euthanized and the articular cartilage/subchondral bone defect site is evaluated histologically for tissue architecture, quantity and quality of the repair.

Example 9

Partial thickness articular cartilage repair model

A partial thickness articular cartilage defect model in the femoral-patellar joint of adult rabbits is used to assess the ability of MIA alone or in combination with BMP or hedgehog protein and carrier to affect cartilage and bone repair. Adult rabbits are anesthetized and prepared for sterile surgery. An up to 4 x 4 mm hole is drilled through articular cartilage into the patellar groove of the knee joint, leaving the underlying subchondral bone intact. The defect is either left empty, filled with the appropriate carrier, or filled with a carrier MIA alone or in combination with BMP or hedgehog protein. Animals are allowed to move freely for four weeks. After four

weeks the animals are humanely euthanized and the articular cartilage defect site is evaluated histologically for tissue architecture, quantity and quality of the repair.

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Patent Claims

1. A pharmaceutical composition containing a melanoma inhibiting activity factor and a biocompatible matrix.

2. A pharmaceutical composition containing a melanoma inhibiting activity factor (MIA) in combination with an osteoinductive protein.

3. A pharmaceutical composition as claimed in claim 2, wherein the ratio of osteoinductive protein : MIA is 1 : 1 to 1 : 20.

4. A pharmaceutical composition as claimed in claim 2 or 3, wherein the osteoinductive protein is BMP-2, BMP-7 or a hedgehog protein.

5. A pharmaceutical composition as claimed in claims 2 to 4, wherein the composition includes a biocompatible matrix.

6. A pharmaceutical composition as claimed in claim 1 or 5, wherein the biocompatible matrix is hyaluronic acid, alginate, collagen, heparin, polylactic-coglycolid and/or polylactic-coglycolid derivatives or combinations thereof.

7. A method for manufacturing a pharmaceutical composition for improved induction of the chondro-/osteogenic lineage and promotion of cartilage and/or bone formation, wherein a melanoma inhibiting activity factor (MIA) is used as the essential component of said composition.

8. A method according to claim 7, wherein the composition contains in addition an osteoinductive protein.

9. A method as claimed in claim 8, wherein the osteoinductive protein is BMP-2 or BMP-7 or a hedgehog protein.

10. A method as claimed in claim 8 or 9, wherein the ratio of osteoinductive protein : MIA is 1 : 1 to 1 : 20.

11. A method as claimed in claims 8 to 10, wherein the melanoma inhibiting activity factor (MIA) is combined with a biocompatible matrix.

12. A method as claimed in claim 11, wherein the biocompatible matrix is hyaluronic acid, alginate, collagen, heparin, polylactic-coglycolid and/or polylactic-coglycolid derivatives or combinations thereof.

13. A pharmaceutical composition containing an expression vector for a melanoma inhibiting activity factor (MIA) or a combination of a vector for the expression of an osteoinductive protein with a vector capable of expression of a melanoma inhibiting activity factor (MIA).

14. A pharmaceutical composition according to claim 13 containing an expression vector for an osteoinductive protein.

15. A method for manufacturing a pharmaceutical composition, wherein an expression vector capable of expression of a melanoma inhibiting activity factor (MIA) or a vector capable of expression of an osteoinductive protein and a vector capable of expression of a melanoma inhibiting activity factor (MIA) is used as the essential component of said composition.

16. A method according to claim 15, wherein the composition contains an expression vector for an osteoinductive protein.

17. A pharmaceutical composition as claimed in claim 13 or 14, wherein the composition includes a biocompatible matrix.

18. The use of a melanoma inhibiting activity factor (MIA) for the treatment of a patient in need of bone and/or cartilage repair.

19. The use according to claim 18, wherein a combination of a melanoma inhibiting activity factor (MIA) and an osteoinductive protein is used.

INTERNATIONAL SEARCH REPORT

[redacted] onal Application No
[redacted]/EP 00/00623

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/57 C07K14/47 A61L27/22 A61L27/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 03328 A (BUETTNER REINHARD ;BOGDAHN ULRICH (DE); KALUZA BRIGITTE (DE); BOEH) 2 February 1995 (1995-02-02) cited in the application page 11; claim 20	1
A	WO 98 30234 A (AIKAWA TOMONAO ;IWAMOTO MASAHIRO (JP)) 16 July 1998 (1998-07-16) cited in the application claims 1,2; examples 1-7	1-10
A	WO 92 09697 A (CELTRIX LAB INC) 11 June 1992 (1992-06-11) cited in the application claims	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 May 2000	07/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	ESPINOSA, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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